

RBRC 00886

Strain name B6;B6C3-Tg (Acro3-EGFP) 010sb

Primer	primer_name	primer_seq	length	Reference
1	acro	GTGGAGCTTTGTGAGGTCACAG	22	Depositor's primer
2		CTTGACAGCTCGTCCATGCCGAG	24	RIKEN BRC
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24	JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25	JAX Genotyping Protocol

Reaction Components

Enzyme TOYOBO KOD FX (Code.No : KFX-101)

Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	60*	*Repeat for 30 cycles

Reagent	Conc	Reaction 1	2	3	4	(μ L)
H2O		0.8				
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/ μ l	1.0				
primer 2	10 pmol/ μ l	1.0				
primer 3	10 pmol/ μ l	1.0				
primer 4	10 pmol/ μ l	1.0				
DNA		1.0				
Taq	1 U/ μ l	0.2				
total		20				

Marker Gene Ladder 100 (Wako, Cat. 316-06951)
Gel 2% agarose in 1 X TAE (100 V, 30 min)



primer_set	product_size	product (allele type)
primer 1	ca 900 bp	Tg
primer 3	324 bp	Internal control (IL-2)

Comment Standard PCR

Novagen KOD Xtreme™ Hot Start DNA Polymerase can be purchased from EMD.

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Strain name B6;C3 Tg(acro3-EGFP)01Osb

Primer	primer_name	primer_seq	length	Reference
1	acro	GTGGAGCTTTGTGAGGTCACAG	22	Depositor's primer
2	gfp	CAGCTTGCCGGTGGTGCAGATG	22	Depositor's primer
3	oIMR0015	CAAATGTTGCTTGTCTGGTG	20	Internal control (Tcrd) JAX Genotyping Protocol
4	oIMR0016	GTCAGTCGAGTGCACAGTTT	20	Internal control (Tcrd) JAX Genotyping Protocol

Reaction Components

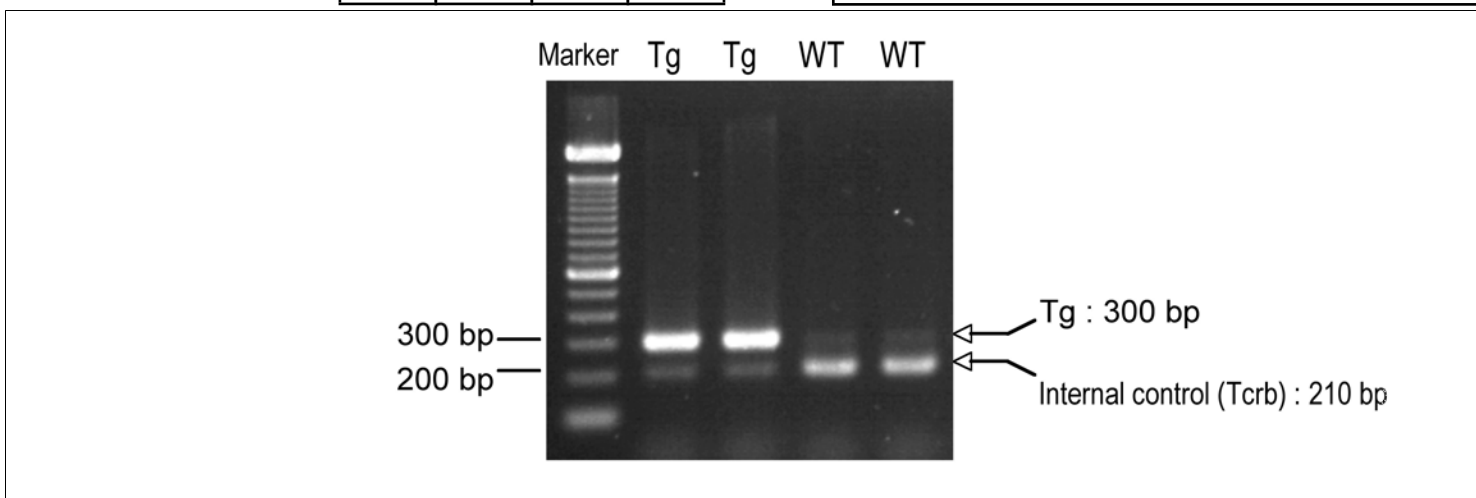
Enzyme Roche Taq (Cat. No. BM1418432)

Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	180	
2	94	30*	
3	60	30*	
4	72	60*	*Repeat for 30 cycles
5	72	300	

Reagent	Conc	Reaction 1	2	3	4	(μL)
H2O		13.3				
PCR buffer	10 X	2.5				
dNTP	2.5 mM	2.0				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.0				
primer 3	10 pmol/μl	2.0				
primer 4	10 pmol/μl	2.0				
DNA		1.0				
Taq		0.2				
total		25				

Marker 100 bp ladder (Invitrogen, Cat. 15628-050)
Gel 2% agarose in 1 X TAE (100 V, 30 min)



primer_set	product_size	product (allele type)
primer 1 primer 2	300 bp	Tg
primer 3 primer 4	210 bp	Internal control (Tcrd)

Comment Standard PCR